IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Guang-Jer Wu

Group Art Unit: 1642

Serial No. 09/653,961

Examiner: S. Rawlings

Filed: September 1, 2000

For: DIAGNOSTIC FOR METASTATIC PROSTATE CANCER

DECLARATION OF GUANG-JER WU UNDER 37 C.F.R. SECTION 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Guang-Jer Wu, hereby declare as follows:

1. I currently hold the position of Associate Professor in the Department of Microbiology and Immunology at Emory University.

2. I have a Ph.D. degree in Biochemistry from the University of California at Davis, California. For the past six years I have carried out and directed cancer research with particular emphasis on understanding the molecular mechanisms underlying carcinogenesis. In particular, my research has included studies on the prostate cancer with a focus of identifying molecular markers useful for predicting the progression of the disease. A copy of my *curriculum vitae* and the list of publications are attached hereto as Exhibit A.

3. I am co-inventor of US Patent Application No. 09/653,961 ("the '961 application") which discloses methods for the diagnosis of metastatic prostate cancer and the prediction of the metastatic ability of prostate cancer in prostate biopsy tissues by measuring the expression levels of MUC18. The claimed invention is based on our findings that the level of expression of a cell surface marker, MUC18, is positively correlated with the metastatic ability of a prostate cancer cell.

4. I have used the claimed method of the '961 application with various clinical samples from 37 patients to determine the relationship between the expression levels of the MUC18 coding sequence and the progression of the prostate cancer. The samples employed in the present study include five different sets representing various disease stages of the tissue, i.e., from normal to metastasis. These include twenty-eight normal epithelium, eleven benign prostatic hyperplasia (BPH), thirty one prostatic intraepithelial neoplasia (PIN), thirty two carcinoma, and five metastatic carcinoma.

5. These samples were obtained from the tissue archive of Emory University Hospital and processed using the protocol optimized for carrying out immunohistochemistry. The antibodies used were made against the recombinant human MUC18 polypeptide representing the amino acid sequence residues of 211 to 376 of the sequence as shown in SEQ ID NO:2 in the '961 application. The details of the protocol can be found in Wu *et al.*, "Expression of a Human Cell Adhesion Molecule, MUC18, in Prostate Cancer Cell Lines and Tissues" (2001) *The Prostate*, **48**:305-315, submitted herewith as Exhibit B.

6. The results of the immunohistochemical studies are summarized in Table 1.

Table 1. Expression of huMUC18 in Normal, BPH, Pre-Malignant PIN, and Malignant Acinar/Ductal Luminal Epithelium of Prostate, and in Metastatic Tumors

Histological type / pathological grade	Number of specimens	Number of cases positive for huMUC18 (+2)	Number of cases positive for huMUC18 (+1)	Percentage of cases positive for huMUC18
Norman epithelium	28	1	2	11
BPH (benign prostatic hyperplasia)	11	0	0	0
PIN	31	18	7	81
Carcinoma (Gleason scores 6-8)**	32	17	10	84
Metastatic carcinoma	5	4		80

** Gleason scores 6-8 refer to the pathological grade of a moderate to poorly differentiated cancer.

7. As seen in Table 1, immunohistochemical staining was negative in 25 out of 28 normal samples and in all 11 BPH samples. Two of the positively scored normal

samples were barely detectable. The +1 indicates a weak staining that is slightly abve the background and the +2 indicates a strong signal that is definitely positive. In contrast, the immunohistochemical staining of pre-malignant PIN, malignant epithelium of prostate, and metastatic tumors were positive in more than 80% of the samples examined. In particular, four of the five metastatic carcinoma samples were stained at the level of +2. These results clearly demonstrate that the expression of the MUC18 coding sequence is indeed increased as the tumor progresses, i.e., there is a positive correlation between the expression levels of the MUC18 and the disease progression from a normal tissue, pre-malignant stage, malignant stage to metastatic carcinoma.

8. Recently, we obtained further data that confirm that the expression of MUC18 could indeed be used as a diagnostic marker for the early detection of the metastatic potential of prostate cancer cells. In these studies, the LNCaP prostate cell line was used because these cells do not express MUC18 and not metastatic. The LNCaP cells were transfected with a DNA construct capable of expressing human MUC18 and three stable cell lines (LNS26, LNS239, and LNS35) expressing high levels of human MUC18 were derived.

9. Next, we examined the effect of MUC18 expression in these MUC18 positive LNCaP cell lines on the cell motility and invasiveness that are art-recognized measurements indicative of the metastatic ability of a cancer cell. As shown in Figure 1, all three cell lines showed increased motility and invasiveness compared to the control LNCaP parent cell line as measured by the procedure of Passatini *et al.* (1992) *Int. J. Cancer* 51:318-324. Further, the increased motility and invasiveness of these cells were dependent on the expression of MUC18 since the antibodies specific for human MUC18 could block these effects.

10. To test the effect of MUC18 expression on the metastasis *in vivo*, cells of the three LNS clonal lines expressing high levels of MUC18 and the control LNCaP line were orthotopically injected into the dorsolateral lobe of prostate glands of male nude mice. After four to five months, we found that the expression of MUC18 was directly responsible for the increased tumor-take of the LNCaP cells in the prostate gland as well as the metastasis of the cells from the prostate gland to the seminal vesicles, the

ureter, the kidney, and the peri-aortic lymph nodes. The results of these studies are shown in Figures 1-6.

Figure 1 shows the effect on huMUC18 expression on the *in vitro* motility (A) and invasiveness (B) of LNCaP cells. The parental LNCaP cells, three huMUC18-expressing clones (LNS26, LNS239, and LNS35), and two vector-transfected clones (LNV49 and LNV51) are used for the experiment according to the procedure described by Passatini *et al.* (1992) *Int. J. Cancer* **51**:318-324. Anti-huMUC18 antibodies (filled columns) or control chicken IgY (gray columns) are used for determination of the effect of anti-huMUC18 antibodies on the *in vitro* motility and invasiveness of these clones.

Figure 2 shows the effect of huMUC18 expression on the establishment of metastatic lesions of the LNCaP cells in various organs of nude mice. (A) shows the discoloration of seminal vesicles after the orthotopical injection of the cells from LNS239 and LNS35 clones, (B) the swollen of the right ureter versus the left ureter as a normal control after the orthotopical injection of the cells from LNS239 clones, (C) the tumor in the right kidney versus the left kidney as a normal control after the orthotopical injection of the cells from LNS26 clones, (D) metastatic lesion at the peri-aortic lymph node in the mouse (on the left) after the orthotopical injection of the cells from LNS239 clones versus no metastatic lesion at the same organ of the control mouse (on the right) after the orthotopical injection of the cells from LNV41 clone, (E) the excised metastatic tumor at the peri-aortic lymph node shown in (D), and (F) discoloration of the right kidney versus the normal left kidney of the same mouse after the orthotopical injection of the cells from LNS35 clone.

Figure 3 shows immunohistochemistry of the prostate tumors induced by the huMUC18-espressing clones in nude mice. The prostate tumors formed after orthotopical injection of the cells from the LNS26 clone were fixed in formaldehyde and embedded in paraffin. **The left panel**: The tumor sections were stained immunohistochemically using the 1/300 dilution of anti-huMUC18 antibodies (A and B) control chicken IgY, or no primary antibody (D). The magnifications are 400X in (A), and 200X in (B)-(D). **The right panel**: The tumor sections were stained immunohistochemically using the 1/3000 dilution of anti-huMUC18 antibodies (A),

the 1/400 dilution of anti huMUC18 antibodies (B), the 1/200 dilution of anti-moMUC18 antibodies (C), or no primary antibody (D). The magnifications are 200X in (A) - (D).

Figure 4 shows immunohistochemistry of the metastatic lesions at the peri-aortic lymph nodes after injection of the huMUC18-expressing clones. The excised metastatic tumors in peri-aortic lymph nodes after orthotopical injection of the cells from LNS239 clone were fixed in formaldehyde and embedded in paraffin. **The left panel**: The tumor sections were stained immunochemically using the 1/3000 dilution of anti-huMUC18 antibodies (A), and the 1/400 dilution of anti-huMUC18 antibodies (B), the 1/300 dilution of anti-moMUC18 antibodies (C), or no primary antibody (D). The magnifications are 200X in (A) - (D). **The right panel**: The tumor sections were stained immunohistochemically using the 1/300 dilution of anti-huMUC18 antibodies (A), the 1/400 dilution of anti-huMUC18 antibodies (B), or no primary antibody (C). Different peri-aortic lymph node metastatic tumor from different mouse was stained immunohistochemically using the 1/300 dilution of anti-huMUC18 antibodies (D). The magnifications were 400X in (A) - (D).

Figure 5 shows the immunohistochemistry of the metastatic lesions at a ureter after injection of huMUC18-expressing clones. The swollen ureter was fixed and paraffin-embedded and stained immunohistochemically. **The left panel**: (A) 1/300 dilution of the anti-huMUC18 antibodies or (B) 1/300 dilution of the anti-moMUC18 antibodies were used. The magnification was at 100X. **The right panel**: (A) 1/300 dilution of the anti-huMCU18 antibodies or (B) 1/300 dilution of the ani-moMUC18 antibodies were used. The magnification was at 400X.

Figure 6 shows immunohistochemistry of the metastatic lesions at the kidneys after injection of the huMUC18-expressing clones. The discolored kidney in Fig. 2F and the kidney with metastatic tumor in Fig. 2C were fixed paraffin-imbedded and stained immunohistochemically. **The left panel**: (A) 1/300 dilution of the anti-huMUC18 antibodies, (B) 1/400 dilution of the anti-huMUC18 antibodies, or (C) 1/300 dilution of the anti-moMUC18 antibodies was used. The magnification was at 200C. **The right panel**: 1/300 dilution of the anti-huMUC19 antibodies was used in

(A)-(C). The magnifications were 100X in (A), 200X in (B) and 400X in (C), respectively.

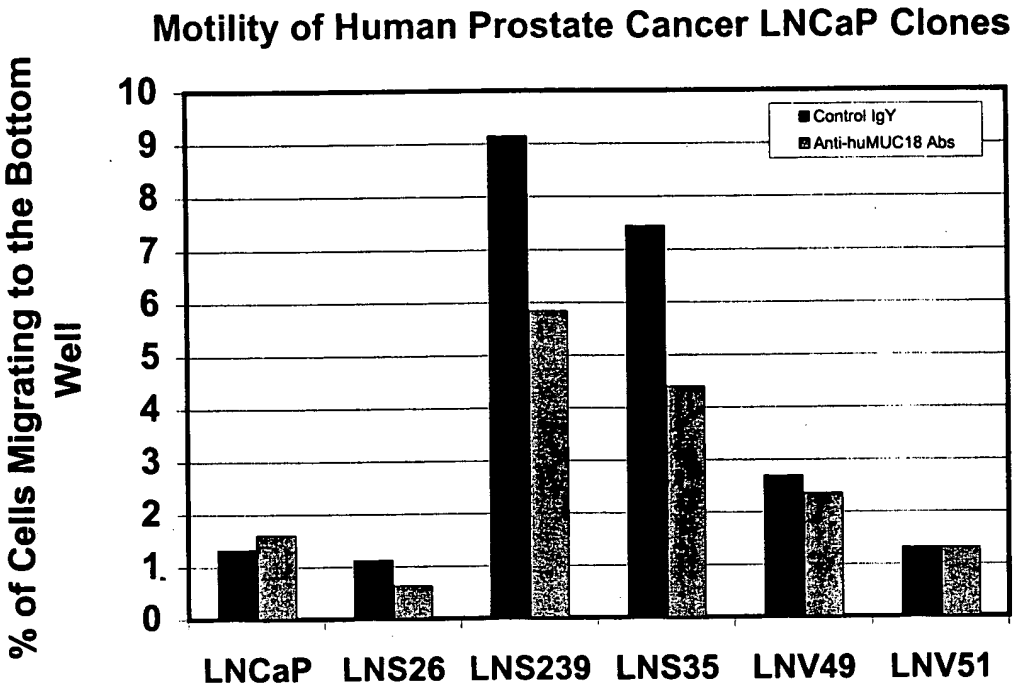
11. In summary, the fact that ectopical expression of MUC18 in LNCaP cells confer the metastatic ability of this non-metastatic cell line *in vitro* and *in vivo* clearly demonstrates that MUC18 plays a critical role in promoting metastasis of the prostate cancer and that MUC18 expression in a prostate cancer cell can be used as a predictor of an increased risk for metastasis of that cancer cell.

12. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believe to be true; and further, that these statements are made with the knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under Section 1001, Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: May 17 2002

Guang-Jer Wu

A


Motility of Human Prostate Cancer LNCaP Clones
% of Cells Migrating to the Bottom Well
10
9
8
7
6
5
4
3
2
1
0
Control IgY
Anti-huMUC18 Abs
LNCaP
LNS26
LNS239
LNS35
LNV49
LNV51

B

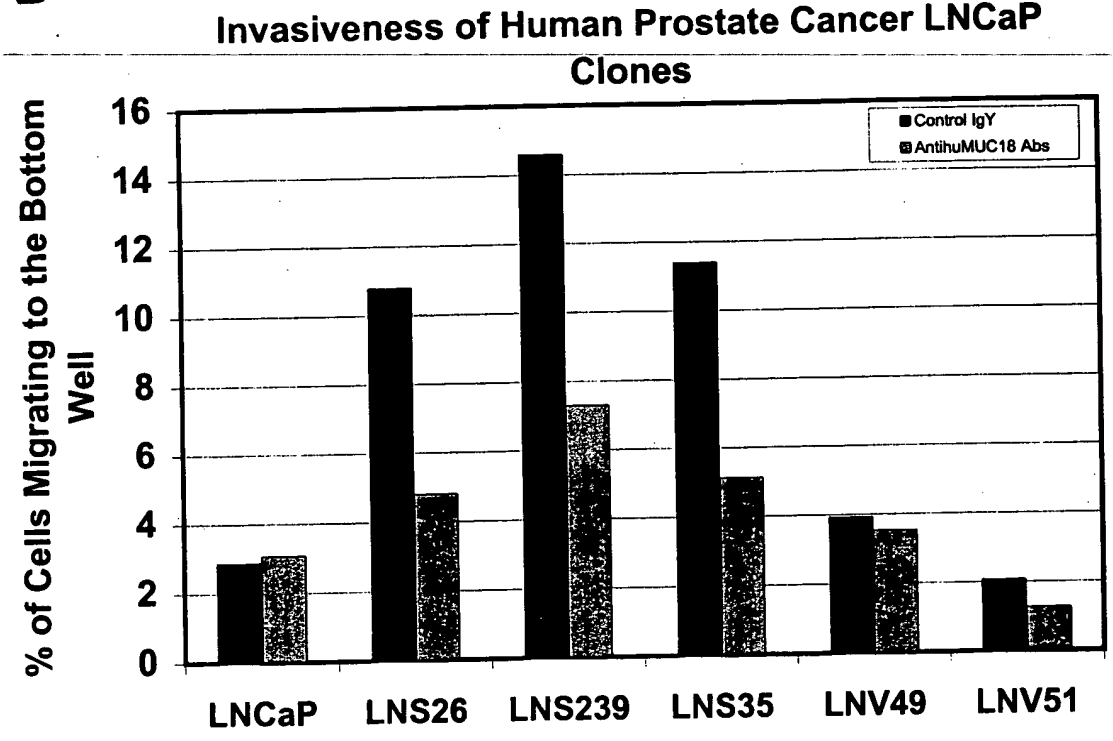
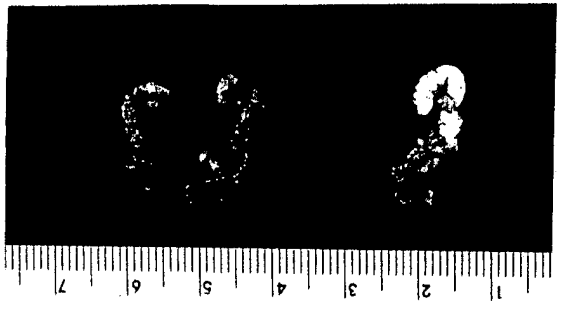
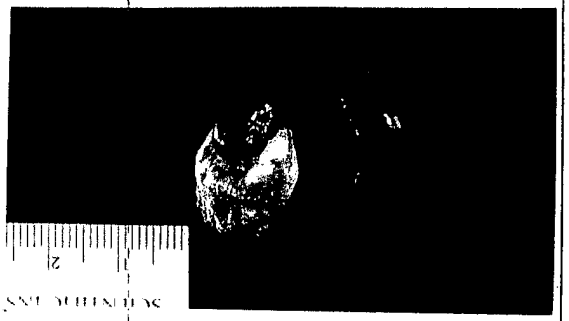

Invasiveness of Human Prostate Cancer LNCaP Clones
% of Cells Migrating to the Bottom Well
16
14
12
10
8
6
4
2
0
Control IgY
AntihuMUC18 Abs
LNCaP
LNS26
LNS239
LNS35
LNV49
LNV51

FIG. 1

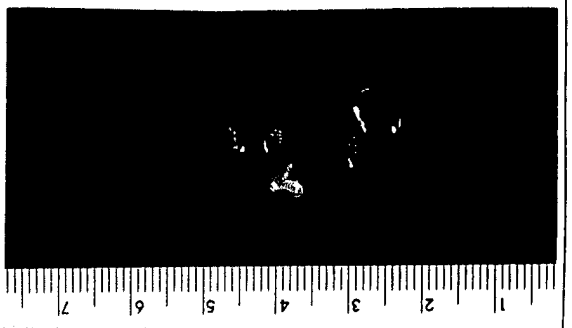
A. Prostate Cancer LNCaP Metastasized to Seminal Vesicles.



B. Prostate Cancer LNCaP Metastasized to ureter.

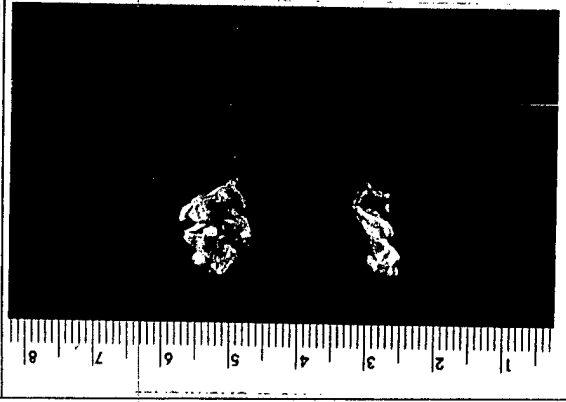


C. Prostate cancer LNCaP Metastasized to Kidney.



D. Prostate Cancer LNCaP and PCa metastasized to Aortic Lymph Node.

E. Prostate Cancer LNCaP metastasized to Aortic Lymph Node.



F. Prostate Cancer LNCaP Metastasized to the Left Kidney.

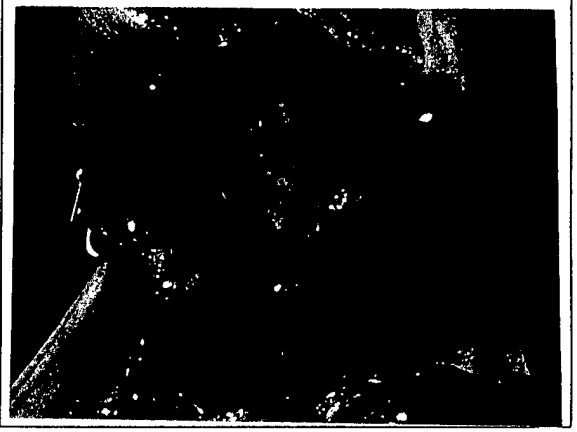


FIG. 2

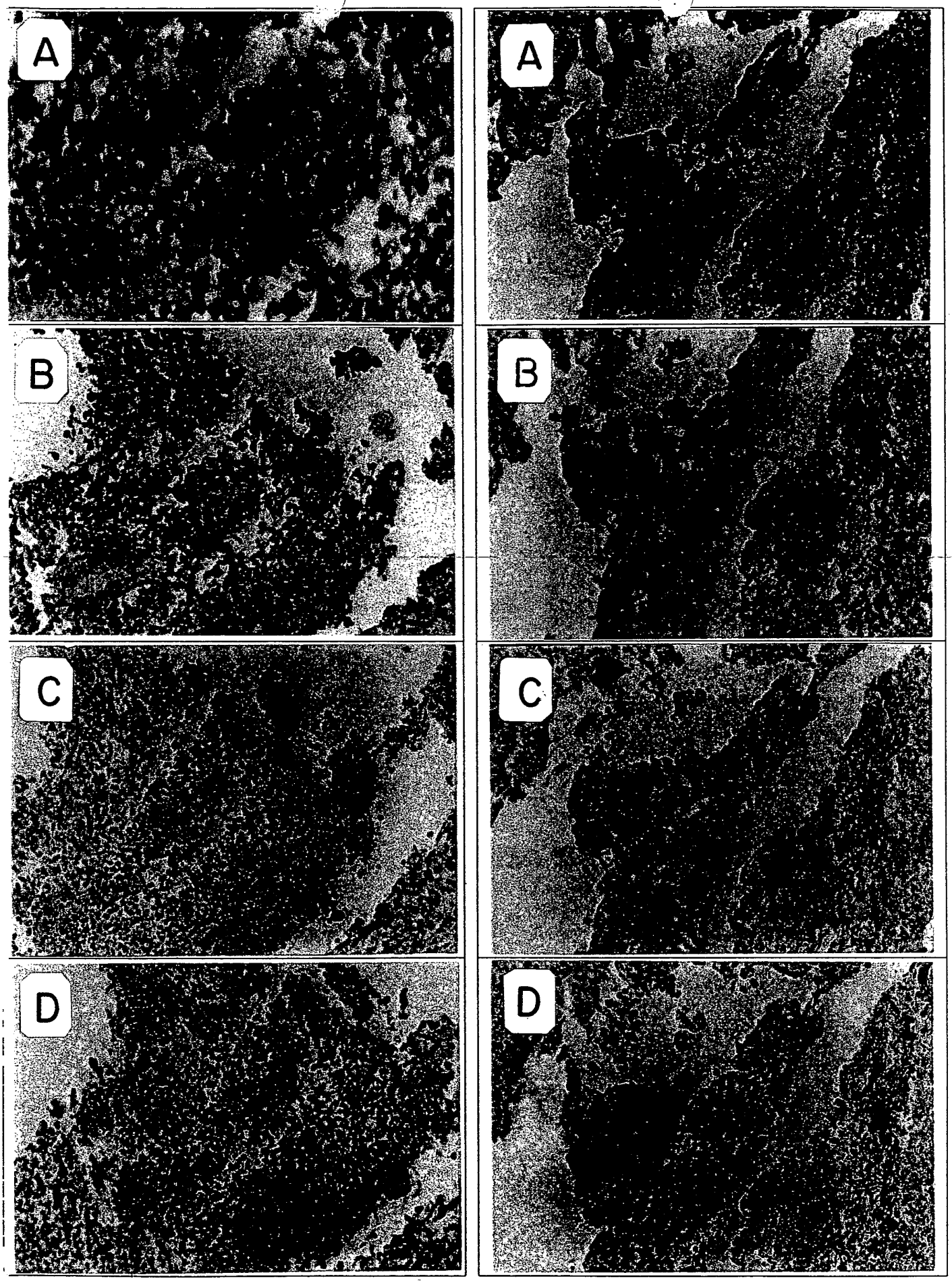


FIG.3

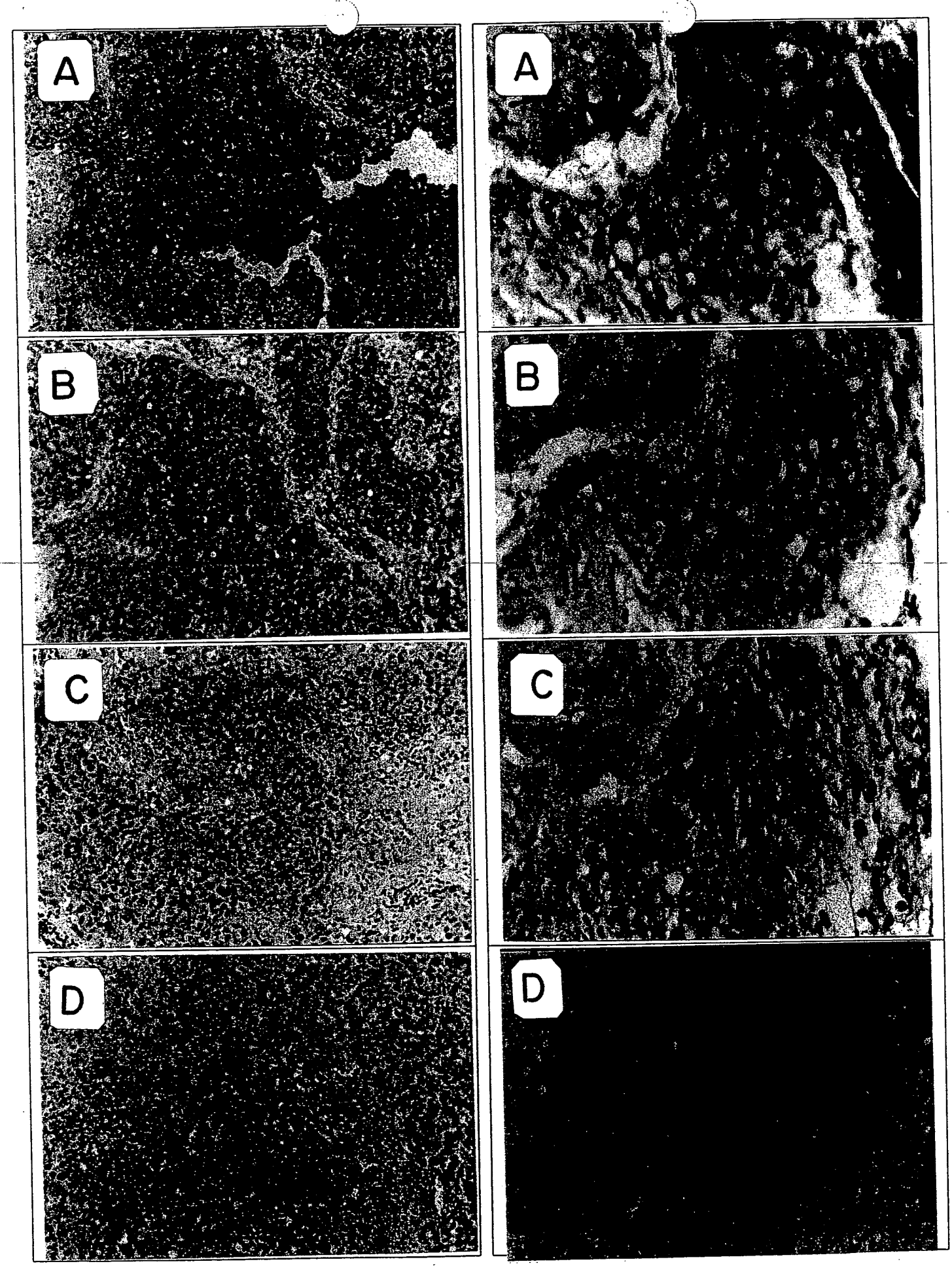


FIG.4

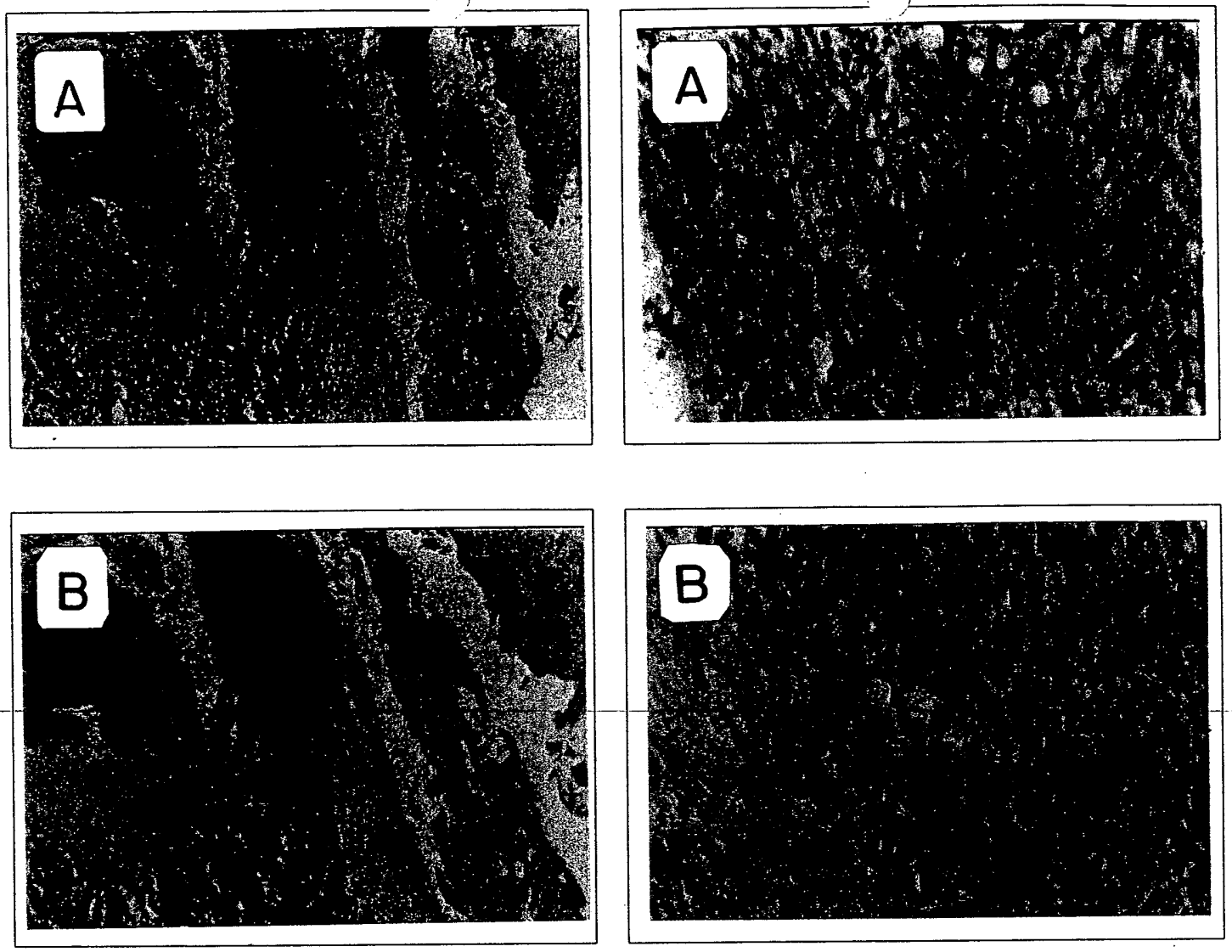


FIG.5

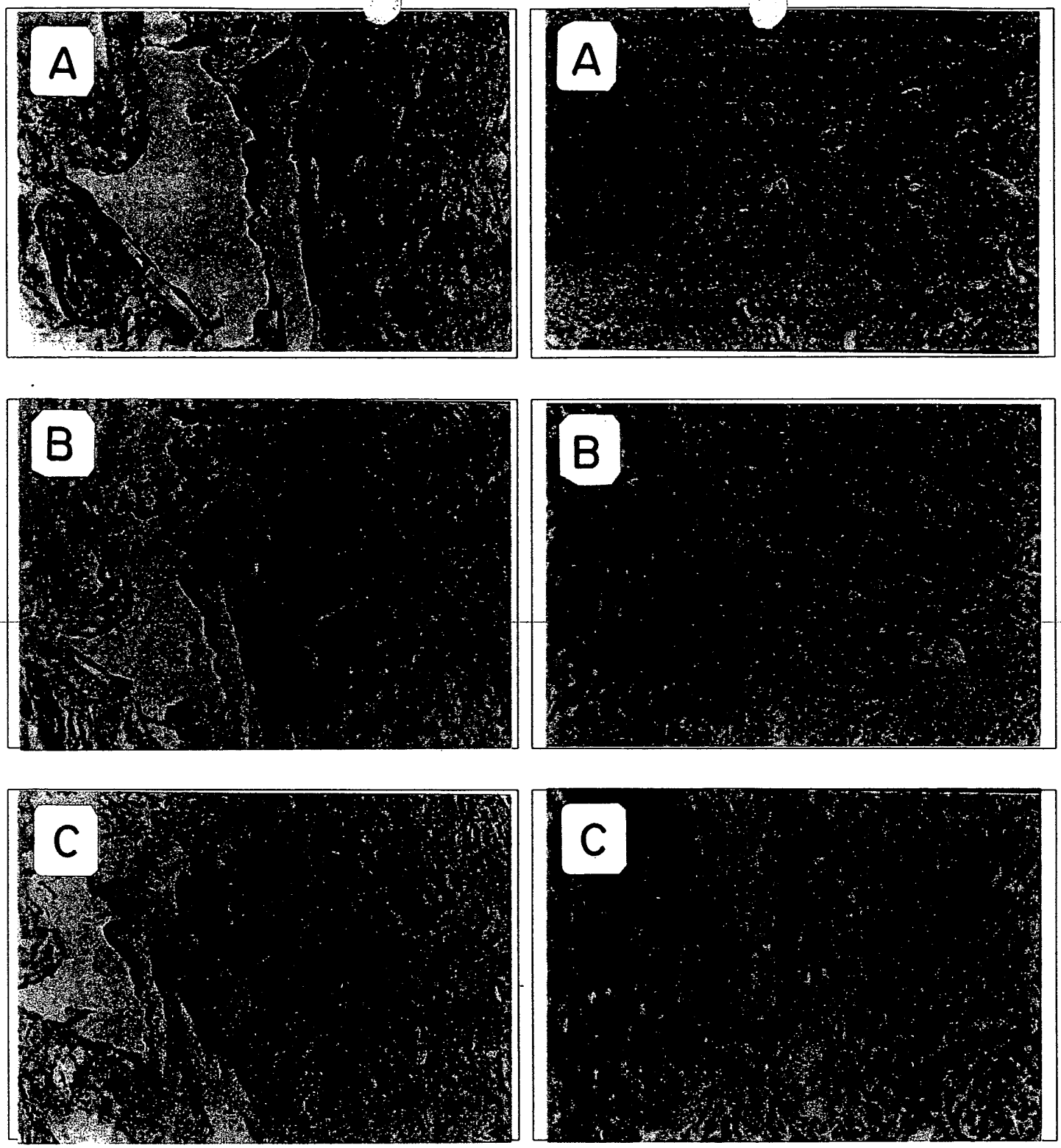


FIG.6

EXHIBIT
A

CURRICULUM VITAE

GUANG-JER WU, Ph.D.

Current Titles and Affiliations:

Primary appointment:
Associate Professor of Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA 30322 (1981-present).

Major research interests:
(a) The role of a cell adhesion molecule, MUC18, in mediating metastasis of prostate cancer and melanoma.
(b) The mechanism of transcriptional regulation of MUC18 gene promoter.
(c) Vaccine studies include DNA vaccines and live recombinant human adenoviruses cancer vaccines.

Joint Appointment:
Faculty member of Winshiop Cancer Center, Emory University School of Medicine (1976-present).

Date of Birth: January 15, 1943

Place of Birth: Taiwan, Republic of China

Citizenship: U.S.A.

Sex: Male.

Marital Status: Married with three children (ages 29, 27 & 20).

Address:

(Office):
Department of Microbiology and Immunology
Rms. 3022 & 3027, Rollins Research Center
Emory University School of Medicine
Atlanta, Ga. 30322
(404)-727-0296 (office)
(404)-727-0295 (laboratory)
(404)-727-3659 (FAX)
Email: wu@microbio.emory.edu.

(Home):
3157, McCully Drive, N.E., Atlanta, GA. 30345
(770)-270-8110.

Education: B.S. - June, 1965 (Oct. 1961 to June 1965)

Department of Agriculture Chemistry
National Taiwan University, Taipei, Taiwan
Major: Agriculture Chemistry
Minor: Microbiology
Thesis Advisor: Dr. Hsi-Hwa Wang.

Ph.D. - December, 1970 (Sept. 1966 to Oct.1970)
Department of Biochemistry and Biophysics
University of California at Davis, Davis, CA.
Major: Biochemistry.
Advisor: Dr. George E. Bruening.

Postgraduate Trainings:

Postdoctoral Fellow (Oct. 1970 to Aug. 1972)

Department of Embryology, Carnegie Institution of Washington, 115, West University PKWY, Baltimore, Md. 21210.

Sponsor: Dr. Igor B. Dawid.

Research project: Isolation and characterization of mitochondrial DNA-dependent RNA polymerase from Xenopus laevis ovaries and transcription of mitochondrial DNA by the enzyme.

Previous Academic and Professional Appointments:

Assistant Professor as a joint appointment (1986 -1995).

Department of Biochemistry, Emory University School of Medicine, Atlanta, GA.

Assistant Professor as a joint appoitment (1976-1980).

Department of Chemistry, Emory University, Atlanta, GA.

Assistant Professor (Aug. 1976 to Aug. 1981)

Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, Ga. 30322.
Major research project:
Regulation of transcription of adenovirus-specific VARNA genes *in vitro*.

Research Associate (Dec. 1972 to July 1976)

Department of Biological Sciences, Columbia University, New York, N.Y.,10027.

Research projects:

Regulation of transcription of adenovirus genes and the rat growth hormone gene.

Research Associate (Sept. 1972 to Dec. 1972)

Institute for Cancer Research, Columbia University, New York, N.Y. 10032.
Research projects :
RNA-dependent DNA polymerase in normal and hepatoma cells.

Teaching Assistant and Graduate Study (Sept.1966 to Oct.1970).

Department of Biochemistry and Biophysics, University of California at Davis, CA. 95616.
Research project: Isolation and characterization of the coat proteins of cowpea mosaic viruses.
Teaching: Advanced Biochemistry Laboratory.

Military Service:

Second lieutenant (ROTC) in Chinese Army, Taiwan, Republic of China (1965-1966).

Committee Memberships: (Institutional)

Emory Representative to University Center (Georgia)/Genetic Task Force (1983-1985).
University microchemical facility committee (1985-1986).
University Biosafety Committee (1986-1992).
University Affirmative Action Committee (1979-present).
Promotion and Tenure Ad hoc committee (1987-1996).
IMP Graduate program Executive Committee (as the graduate student recruiter) (1994-1996).
Ph.D. student Thesis Committees: 1983-1997.

- Bey-Dih Chang (Barry Warner) (1983-1990).
- Celeste Ray (Charles Moran) (1983-1987).
- Terry Keeney (Charles Moran) (1983-1987).
- Sue Hasegawa (Jerry Boss) (1989-1992).
- Jim Riley (Jerry Boss) (1992-1993).
- Andrew Chung (Doug Wallace) (1990-1993)
- Ingird Ruf (Dan Rawlins) (1990-1996).
- Gloria Hsieh (George Jones) (1991-1993).
- Yu-Sheng Chen (Doug Wallace) (1991-1997).
- Ted Seyler (Charles Moran) (1993-1997).
- LiTing Cheng (Richard Compans) (2002-present).

Departmental Services:

Polaroid film facility (1990-1993).
Beckman Oligo1000 DNA synthesizer (1993-present).

Grant reviewer: (Institutional and International).

University Research Committee (1984, 1988, 1990, 1991).
Israel Academy of Science and Humanity/Basic Research Foundation (1991).
IBMS/Academia Sinica/Taiwan/Clinical Research Center Fund (1990, 1991, 1992, 1993, 1994).

Manuscript reviewer: (Ad hoc)

Cell, 1981
J. Biol. Chem. 1992.
Virus Research, 1996, 1998, 2001.

Publicity and press conference:

Jan. 27, 1980. New York Times & Chattanooga Times, "New genes discovery may alter research".

Honor and Awards:

Virtue, Wisdom, Health and Conduct award, National Taiwan University, 1963-1965.
Honor equivalent to summa cum laude, graduated at the top of a class of fifty-five, Department of Agriculture chemistry, National Taiwan University, 1965.
Daymon-Runyon foundation postdoctoral fellowship, Columbia University, 1973-1975.
National Science Foundation grant award, $ 115,000 (100%) Co-PI, (Dr. G. Zubay as PI), 1975-1978.
National Cancer Institute grant award, $183,000, PI, 90%,1979-1982.
National Science Foundation grant award, $100,000 (10%) Co-PI, (Dr. Ray Shapira as PI), 1980-1983.
Two University research awards, 1977 and 1982.
Two Institutional Biomedical Research Support Grants, 1982 and 1983. $15,000 each.
Rockefeller Foundation grant award, $21,000, PI, 1985-1986.
University Research Grant (a merit award), $9,000, 1987.
National Institutes of Health grant award, $227,767, PI, 1986-1989.
Institutional Biomedical Research Support Grant, $10,000, 1989-1990.
Emory Cancer center ACS seed grant (a merit award), $8,000, 1991-1992.
University Research grant (a merit award), $10,000, 1992-1993.
National Institutes of Health grant, $199,950, (15%) Co-PI, (Larry Phillips as PI),1992-1996.
Winship Cancer Center melanoma seed grant, $15,000, 1996-1997.
National Institutes of Health grant, $240,000, (10%) Co-PI, (Larry Phillips as PI), 1997-2000.

Emory skin diseases research center-pilot and feasibility grant, $15,000, 1997-1998.
Emory University Research Committee grant, $19,000, 1998-1999.
NCI Developmental grant for prostate cancer research, $100,000, (30%), PI, 3/1/99-7/31/00.
NIH, CFAR core facility of AIDS research, $35,000-65,000, Co-PI (20%) 1999-2001.
Department of Defense, US Army grant for Prostate cancer, $110,000 per year, 3/1/00-2/28/03, 40% (PI)

Society Memberships:

American Association for the Advancement of Science, 1966-present.
American Society for Microbiology, 1972-present.
American Society for Virology, 1982-1996.
Society of Chinese Bioscientists in America, 1987-present.
American Society for Biochemistry and Molecular Biology, 1986-present.
American Associaton for Cancer Research, 1977-1980, 1999 -present.

Patents:

Emory File No.97056, Provisional Patent for "Diagnostic for metastatic prostate cancer".

Grant support: (Currently active and pending supports)

(a) Active

(i) Department of Defense, US Army Grant, $100,000 per year, 3/1/00-2/28/03, 40% (PI). "MUC18, a mediator and marker of prostate cancer metastasis."
The major goal of this project is to study the role of MUC18 in prostate cancer metastasis.

(ii) Pfizer Inc., $15,000 for one year, 7/1/02-6/30/03, 5% (PI). "Effect doxazosin on the growth and metastasis of prostate cancer."
The major goal of this project is to study the effect of doxazosin on the huMUC18-mediated tumor growth and metastasis of prostate cancer LNCaP cells in a nude mouse model.

(b) Pending.

Department of Defense, US Army Idea Grant, $175,000 per year, 4/1/03-2/28/06, 50% (PI), "Mechanism of MUC18-mediated development and malignant progression of human prostate cancer."

The major goal of this project is to study the mechanism of MUC18-mediated development and malignant progression of human prostate cancer.

Formal teachings:

(a) Medical Profession teachings:

Medical Microbiology (medical students): 1978-1990.
Medical Microbiology (dental and nurse students): 1980-1982.
Laboratory exercise of Medical Microbiology (medical students): 1988-1993.
Medical Microbiology (physician assistants): 1990-present.

(b) Graduate School teachings:

Molecular biology (723): 1980 - 1986.
Human Genetics: 1987-1992.
Introduction of Animal virology (IBS513): 1979, 1982, 1983, 1984, 1986, 1987, 1992, 1994, 1996, 1997, 1998, 1999, 2000, 2001-present.
Introduction to Research: 1989-present.
Molecular Animal Virology (IBS723): 1989 - 1995.
Molecular Biology of Cancer Metastasis (IBS 562): 1999-present.

Supervisor teachings:

(a) Graduate students:

Vaughn Kubiak, 1976-1978, M.S., currently as a large animal product manager, Charles city, Iowa.
Thomas Dechiara, 1977-1980, M.S., currently as a posdoctoral scientist, N.J.
Helen Lindsey-Ramsey, 1976-1983, Ph.D., currently as an administrator at CDC, Atlanta, Ga.
Shiu-Ying Lu, 1977-1983, Ph.D., currently as a church worker at Houston, Tx.
Johnny F. Railey, Jr., 1981-1987, Ph.D., currently as a junior faculty at National Patent Office, Arlington, VA.
Ronald E. Cannon, Ph.D., 1979-1985, Ph.D., currently as a junior faculty at National Institute of Environmental Health Sciences, Research Triangle, N.C.
Claudiu I. Bandea, 1984-1990, Ph.D., currently, as a senior scientist at CDC.
David W. Digby, 1989-1996, Ph.D., currently as a postdoctor at Clark University, Atlanta, GA.

(b) Postdoctor fellows:

Larry L. Low, Ph.D., (1982-1983) currently as a Scientist at Atlanta University, Atlanta, Ga.

Kazuaki Mannen, M.D. (1983-1984) currently as an Associate Professor at Oita Univ, Japan.
Yue-Fang Xue, Ph.D. (1989) currently as an Associate Professor at Beijing University, China.
Shu-Ling Lien, Ph.D., (1988-1989) currently as a respiratory therapist, Washington D.C.
Kai Sun, Ph.D., (1996-1997) currently as a postdoctor at Harvard University, Boston, MA.
Yue Wu, M.D. (11/1/96 to 3/14/97) currently as a postdoctor at Neuroscience, Emory University, Atlanta, Ga.
Hsiuchin Yang, Ph.D. (9/1/96 – 4/30/98) currently as a Lab chief at Dept of Biology, Georgia State University, Atlanta, Ga.
Peng-peng Qu, M.D. Ph.D. (7/1/98-2/28/99) currently as a medical staff and postdoctoral fellow at Tianjin University, PRC.
Zhong Lu, M.D. (3/15/99-3/15/00) currently as a technician in Canada.
Qiong Peng, M.D., Ph.D. (10/1/99-9/15/00) currently as a postdoctor at Goergia Inst Tech.
Wenping Sun, M.D. (6/1/00-9/15/00) currently as a research associate at Dept of Biotechnology, Emory University.
Pingping Fu, M.D. (5/1/01-2/28/02) currently as a postdoctor at the Winship Cancer Institute, Emory University.
Shur-wern Chris Wang Chern, Ph.D (9/1/98-2/15/99, and 8/15/00-present).
Cheng-Feng Frank Chiang, Ph.D. (4/19/02-present).

Lectureships, Seminar Invitations, and Visiting Professorships:

Visiting Scientist, Institute of Biomedical Sciences, Academia Sinica, Feb.- Aug., 1989, "Stable expression of the human CMV IE genes in HeLa cells".
Invited seminar, Institute of Molecular Biology, Academia Sinica, Feb. 11, 1992, "Transcriptional control of the VARNA1 gene".
Invited seminar, Department of Cell Biology, Baylor Medical college, Houston, TX, May 6, 1999, "Expression of MUC18 and metastasis of melanoma and prostate cancers".
Invited seminars, MUC18 and melanoma and prostate cancer metastasis", NHRI and Institute of Biomedical Sciences, Academia Sinica, Oct 9, 2000; Veterans General Hospitals, Taipei, Taichung, and Kaohsiong, Taiwan, Oct. 7, 9, & 12, 2000, Department of Microbiology and Immunology, Tzu-Chi College of Medicine, Hualien, Taiwan, Oct 11, 2000.

Invitation to National or International Conferences:

International Symposium of "Structure and Function of Nucelic Acids and proteins, " at IBMS, Academia Sinica, Taiwan, May 11-13, 1989.
SCBA symposium, at Chinese university, Hong Kong, June 24-30, 1990.

Other activities:

(a) Religious activities (Christian):

Deacons of Atlanta Chinese Christian Church (ACCC), 1980-1985, 1990-1994.
Superintendent of Adult Sunday School, ACCC, 1985-1988.
Chairman of Joint Elder-Deacon Board of ACCC, 1991-1993.
Adult Sunday School Teacher (Chinese and English), ACCC, 1980-present.
Central District Home-fellowship Consulor, ACCC, 1993-present.
Emory University Chinese Christian Fellowship Consulor, ACCC 1995-present.
Lay-preacher of Chinese Christian Fellowships and Churches in the Southeastern Region of USA, 1993-present.
Elder-elect of ACCC, 1996-1997.
Elder of ACCC, 4/13/1997-present.

(b) Non-religious activities:

Toastmasters International, member, 1996-present.
Competent Toastmaster (CTM), Sept/22/1997.
Able Toastmaster bronze (ATM-B), July/1999.
Club president, TM Clifton Corridor Club #7165, District #14, 7/1/1997-6/30/1998.
Vice president of Membership, TM Clifton Corridor Club, 7/1/98-present.

Publications:

1. **Wu, G.-J.** and G. E. Bruening, Two proteins from cowpea mosaic virus. Virology (1971) 46, 596-612.

2. **Wu, G.-J.** and I.B. Dawid, Solubilization of mitochondrial RNA polymerase from ovaries of Xenopus laevis. Carnegie Institution Year Book (1971) 70, 46-47.

3. **Wu, G.-J**. and I.B. Dawid, Isolation and properties of mitochondrial RNA polymerase from ovaries of Xenopus laevis. Carnegie Institution Year Book (1972) 71, 25-29.

4. **Wu, G.J.** and I.B. Dawid, The purification and properties of mitochondrial RNA polymerase from ovaries of Xenopus laevis. Biochemistry (1972) 19, 3589-3595.

5. **Wu, G.J.** and I.B. Dawid, In vitro transcription of Xenopus laevis mitochondrial DNA by homologous mitochondrial RNA polymerase. J. Biol. Chem.(1974) 249, 4412-4419.

6. Dawid, I.B. and **G.-J. Wu**, Transcription of mtDNA by mitochondrial RNA polymerase from Xenopus laevis, in " The biogenesis of mitochondria," (Kroon, A.M. and Saccone, E., eds.) (1974) pp. 79-84, Academic Press.

7. Dawid, I.B. and **G.-J. Wu**, Transcription of mitochondrial DNA by mitochondrial RNA polymerase from xenopus laevis, Carnegie Institution Year Book (1973) 72, 46-47.

8. Bancroft, F.C., **Wu, G.-J.** and G. Zubay, Cell-free synthesis of rat growth hormone, Proc. Natl. Acad. Sci. U.S.A. (1973) 70, 3646-3649.

9. **Wu, G.-J.** and G. Zubay, Prolonged transcription in a cell-free system involving nuclei and cytoplasm, Proc. Natl. Acad. Sci. U.S.A. (1974) 71, 1803-1807.

10. **Wu, G.-J.**, Luciw, P., Mitra, S., Zubay, G. and H. Ginsberg, in vitro approaches to the study of adenovirus transcription, in " Nucleic Acids-Protein Recognition ", Ed. by H. Vogel, (1977) pp. 171-186, Acad. Press, New York.

11. **Wu, G.-J.**, Adenovirus DNA directed transcription of 5.5 S RNA in vitro, (1978) Proc. Natl. Acad. Sci. U.S.A. 75, 2175-2179.

12. **Wu, G.-J.**, Faithful transcription of adenovirus 5.5 S RNA gene by RNA polymerase III in a human KB cell-free extract,(1980) J. Biol. Chem.255, 251-258.

13. Cannon, R.E., Tatti, K.M. and **Wu, G.-J.**, A rapid, small scale procedure for the preparation of plasmid DNA for screening mutants by the dideoxyribonucleotide chain-terminator sequencing method, (1985) BioTechniques 3, 93-96.

14. **Wu, G.-J.**, Lu, S.-Y.,Lowe, L.L., and Kinkade, Jr., J.M., Identification of lactate dehydrogenase-M polypeptide translated in vitro from human and mouse tumor cell poly(A)-containing mRNA, (1985) Int. J. Biochem. 17, 353-363.

15. Hess, J., Perez-Stable, C., **Wu, G.-J.**, Weir, B., Tinoco, I. Jr., and Shen, James C.-K., End-to-end transcription of an Alu-family repeat: a new type of RNA polmerase III-dependent terminator and evolutionary implication, (1985) J. Mol.Biol. 184, 7-21.

16. **Wu, G.-J.** and Cannon, R.E., An economical large scale procedure to purify E. coli amplifiable plasmids for DNA sequencing, in vitro transcription and in vitro mutagenesis, (1985) Experientia 41, 1488-1490.

17. Cannon, R.E., **Wu, G.-J.** and Railey, J.F., Functions of and interactions between the A and B blocks in Ad2 specific-VARNA1, (1986) Proc. Natl. Acad. Sci. U.S.A. 83, 1285-1289.

18. **Wu, G.-J.** and Cannon, R.E., Termination sequences in the control region of AD2 specific VARNA2 gene,(1986) J. Biol. Chem.261, 12633-12642.

19, **Wu, G.-J.**, Book Review," Adenovirus DNA: The Viral Genome and its Expression" (ed. by W. Doerfler) (1986) ASM News 52, 552-553.

20.**Wu, G.-J.**, Railey, J.F., and Cannon, R.E., Defining the functional domains in the control region of Ad2 specific-VARNA1 gene, (1987) J. Mol. Biol. 194, 423-442.

21.Railey, J.F. and **G.-J. Wu**, Organization of the regulatory elements in the control region of the Ad2 specific-VARNA1 gene: fine mapping with linker-scanning mutants, (1988) Mol. Cell. Biol. 8, 1147-1159.

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24.**Wu, Guang-Jer**, Formation of large, sedimentable transcription complexes with VARNA genes and other-related genes, (1989) J. Biol. Chem. 264, 9753-9761.

25.Wu, C.-W., Sierra, E. E., Daly, T. J., **Wu, G.-J**. and Wu, F. Y.-H., Structural and functional studies of Xenopus transcription factor IIIA, in "The Eukaryotic Nucleus: molecular biochemistry and macromolecular assemblies" (ed. Samuel H. Wilson), Telford Press. (1989) pp.441-461.

26.**Wu, G.-J.**, Bandea, C. I. and Wu, Mei-Whey H. Transcriptional control of the adenovirus specific VARNA1 gene, in "Structure and function of nucleic acids and proteins", (eds. F. Y.-S. Wu and C.-W. Wu), (1990) Raven Press, pp.185-213.

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31.**Wu, G.-J.**, Bandea, C. I., Naiquan Yang, Lien, S.-L., Wu, M.-W. H., Hsieh, Gloria C.-J., and Lu, F. Transcription function of each base pair in the control region of the adenovirus VARNA1 gene (1994) Virology 200, 105-113.

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43. **Wu, G.-J.**, Wu, M.-W.H., Wang, S.-W., Liu, Z., Peng, Q., Qu, P., Yang, H., Varma, V. A., Sun, Q., Petros, J. A., Lim S., and Amin, M. B. (2001) Isolation and characterization of the major form of human MUC18 cDNA gene and correlation of MUC18 over-expression in prostate cancer cells and tissues with malignant progression". Gene 279, 17-31.

44. **Wu**, Guang-Jer, Peng, Q., Fu, P., Wang, S. -W. Chris, and Wu, M. –W. H., (2002) MUC18 is required for the tumorigenesis and metastasis of human prostate cancer LNCaP cells. The Proceedings of the 93rd Annual meeting of the American Association for Cancer Research, San Francisco, Calif., April 6-10, 2002, P. 1065, #5277.

45. **Wu, G.-J.**, Peng, Q., Fu, P., Wang, S.-W. C., Dillehay, D. L., and Wu, M.-W. H. (2001) MUC18 expression increases both tumor-take and metastasis of prostate cancer LNCaP cells. (submitted).

46. **Wu, G.-J.**, Peng, Q., Fu, P., Wang, S.-W. C., Yang, H., Dillehay, D. L., and Wu, M.-W. H. (2001) Effect of MUC18 expression on in vitro invasiveness, tumorigenesis, and metastasis of murine melanoma cells in syngeneic mouse model. (in preparation).

47. **Wu, G.-J.**, Fu, P., Wang, S.-W., and Greenberg, N. (2001) Expression of MUC18 correlates with metastasis of prostate adenocarcinoma in the (TRAMP) transgenic adenocarcinoma mouse prostate model. (in preparation).

Description of current research activities:

This laboratory focuses on the molecular biology of prostate cancer and melanoma metastasis. Over-expression of a cell adhesion molecule, MUC18 (CD146, MEL-CAM), has been correlated with the metastatic propensity of melanoma cells. To study its role in mediating metastasis, we have used RT-PCR to amplify the human MUC18 cDNA and used RACE and RT-PCR to amplify the mouse MUC18 cDNA. The

DNA sequence and the deduced amino acid sequence of the mouse MUC18 cDNA is about 74% identical to the human MUC18 cDNA. The amino acid sequence of human MUC18 cDNA is similar to the published sequence except 7 amino acids. We have inserted both the huMUC18 and muMUC18 cDNA genes into a mammalian expression vector and a bacterial GST-fusion protein expression vector. We have purified the bacterially expressed proteins and generated polyclonal antibodies in chickens. We have transfected an expressible muMUC18 cDNA gene into a non-tumorigenic and non-metastatic melanoma cell line, K1735-clone 10, and a tumorigenic and non-metastatic melanoma cell line, K1735-clone 3, and obtained G418-resistant clones that express high levels of MUC18. We inject the cells from the MUC18-high-expression clone derived from K1735-10 into syngeneic mice via *i.v.* and *s.c.* routes to study their pulmonary metastasis *in vivo*. We found this clone, but not the three control clones, could cause pulmonary metastasis via the *i.v.* route. But the pulmonary metastasis by this clone was inefficient (about 33% of mice) and only microscopic nodules were formed. We also inject the cells from the MUC18-high-expression clone derived from K1735-3 into syngeneic mice via *i.v.* and *s.c.* routes to study their pulmonary metastasis *in vivo*. We found this clone, but not the three control clones, could cause pulmonary metastasis via the *i.v.* route. The pulmonary metastasis by this clone was very efficient (100% of mice) and the pulmonary nodules were numerous and very large with a diameter of 2-5 mm. We also obtained G418-resistant clones that express high levels of MUC18 from another tumorigenic and non-metastatic murine melanoma cell line K1735-clone 9 and tested the effect of MUC18 expression on melanoma growth *in vivo* via *s.c.* route of injection. We found that expression of MUC18 suppressed the tumor growth. All the muMUC18-high expressing clones from all three K1735-clones caused minimal pulmonary metastasis via the *s,c.*route. We conclude that muMUC18 expression definitely promotes melanoma cells to have pulmonary metastasis, but the effect is dependent upon the intrinsic properties of the melanoma cells. Mouse MUC18 expression may have a negative effect on melanoma growth *in vivo*. Taken the above results together, we conclude that muMUC18 expression may mediate later steps of, but not early steps of melanoma pulmonary metastasis that required cofactors. Currently we are in the process of defining the functional domains and the ligands and cofactors of muMUC18.

We also studied the expression of human MUC18 in prostate cancer cell lines and tissues. We found that huMUC18 only expressed in metastatic prostate cancer cells, but not in non-metastatic cancer cells. Human MUC18 was weakly expressed in normal prostate gland and the derived normal epithelial cells in culture. HuMUC18 was highly expressed in 80% of the pre-cancerous and cancerous prostate tissues. The level of MUC18 expression appeared to increase with increasing pathological grades. We have proposed that MUC18 may also mediate metastasis of prostate cancers. To test this hypothesis, we transfected mammalian cells-expressible huMUC18 cDNA gene into the LNCaP cells that have been shown to express no MUC18. The huMUC18-high-expressing LNCaP clones were injected orthotopically into the dorsolateral lobe of the nude mice prostate gland. We found that huMUC18 expression in these LNCaP clones could mediate increasing tumor take as well as metastasis to the seminal vesicles, the peri-aeortic lymph node, ureter, and kidney. This shows that MUC18 plays an important role in causing human prostate cancer cells to metastasize other organs, if they are injected orthotopically, but not subcutaneously. Currently we are in the process of

defining the functional domains and the ligands and cofactors of huMUC18. We have been collaborating with Norman Greenberg on his TRAMP model. When the prostate cancer cells in the TRAMP male mice started to metastasize to other organs at about the age of 200 days and beyond, MUC18 expression was detectable and increased in the tumor developed in the mouse prostate gland. Thus MUC18 expression is increased during malignant progression of prostate cancer in this transgenic mouse model. This result further corroborates our hypothesis above. We are also collaborating with Leland Chung's group on a bone metastasis xenograft model, and with Chris Gregory and Thomas Pretlow on their CWR22 xenographft model. We will also collaborate with Jeff Gordon on his neuroendocrine cells-derived prostate carcinoma transgenic mouse model. We have also cloned the genomic copies of these genes that contain the 5'-flanking transcription regulatory sequences for studying transcription factors and signal transduction mediators that regulate their expression in normal versus cancer cells. Methods used for the research include recombinant DNA methods, cell cloning, DNA sequencing, PCR, immunoblotting, Northern and Southern blotting, immunohistochemistry, gene expression, protein-DNA interactions, tissue culture, and mouse models.